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Viral Serologies in Patients With Chronic Fatigue and Chronic Fatigue Syndrome

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Chronic fatigue syndrome (CFS) is an illness characterized by disabling fatigue associated with complaints of fevers, sore throat, myalgia, lymphadenopathy, sleep disturbances, neurocognitive difficulties, and depression. A striking feature of CFS is its sudden onset following an acute, presumably viral, illness and the subsequent recurrent "flu-like" symptoms. It has been speculated that both CFS and debilitating chronic fatique (CF) that does not meet strict criteria for CFS may be the direct or indirect result of viral infections. We therefore tested 548 chronically fatigued patients who underwent a comprehensive medical and psychiatric evaluation for antibodies to 13 viruses. Our objectives were to compare the seroprevalence and/or geometric mean titer (GMT) of antibodies to herpes simplex virus 1 and 2, rubella, adenovirus, human herpesvirus 6, Epstein-Barr virus, cytomegalovirus, and Coxsackie B virus, types 1–6 in patients with CF to healthy control subjects. Other goals were to determine if greater rates of seropositivity or higher GMTs occurred among subsets of patients with CFS, fibromyalgia, psychiatric disorders, a self-reported illness onset with a viral syndrome, and a documented temperature >37°C on physical examination. Differences in the seroprevalence or GMTs of antibodies to 13 viruses were not consistently found in those with CF compared with control subjects, or in any subsets of patients including those with CFS, an acute onset of illness, or a documented fever. These particular viral serologies were not useful in evaluating patients presenting with CF.

KEY WORDS: chronic fatigue syndrome, chronic fatigue, fibromyalgia, Coxsackie B, herpesviruses, viruses

INTRODUCTION

In the past decade, there has been a growing interest in conditions which share the symptom of persistent, disabling fatigue. It has been speculated that several of these conditions, for example, chronic fatigue syndrome (CFS), or debilitating chronic fatigue (CF) that does not meet criteria for CFS, may be the direct or indirect result of chronic viral infections. CFS is an illness characterized by severe fatigue associated with fevers, sore throat, myalgia, lymphadenopathy, sleep disturbances, neurocognitive difficulties, and depression [Holmes et al., 1988]. One of the most striking features of CFS is its sudden onset, often following an acute, presumably viral, illness [Komaroff and Buchwald, 1991]. Thereafter, patients commonly report symptoms of a recurrent "flu-like" illness. Up to 70% of patients with CFS and CF also meet criteria for fibromyalgia (FM) [Goldenberg et al., 1990; Buchwald et al., 1994], a common rheumatologic condition typified by musculoskeletal pain and the presence of soft tissue "tender points" [Wolfe et al., 1990]. FM, like CFS, frequently follows a self-reported viral illness [Buchwald et al., 1987; Wysenbeek et al., 1991].

The prominence of the acute onset of illness, the persistent symptoms consistent with a viral infection, and early reports of increased titers of viral antibodies and enhanced activity of the interferon-induced enzyme, 2′5′-oligoadenylate synthetase [Straus et al., 1985; Morag et al., 1982], has led to the examination of the role of viruses in CFS and CF. Despite an extensive search, conclusive evidence of a direct link between viruses and CFS and CF has not been found and viral serology is not considered to be helpful in evaluating most patients [Hellinger et al., 1988; Buchwald et al., 1987; Holmes et al., 1987; Landay et al., 1991]. However, previous studies often assessed only a single virus or, more importantly, did not identify subsets of patients who might be more likely to have evidence of infection. To further examine the usefulness of viral serologies in the evaluation of chronically fatigued patients, and to clarify the role of viruses in patients with CFS, we performed serologic tests on individuals seen in a referral clinic. Our objectives were to describe the seroprevalence

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of herpes simplex virus type 1 (HSV-1), type 2 (HSV-2), and rubella, and to determine if higher levels of antibody were present to adenovirus, human herpesvirus 6 (HHV-6), Epstein-Barr virus (EBV), cytomegalovirus (CMV), and Coxsackie B virus (types 1–6), in chronically fatigued patients compared to healthy control subjects. Other goals were to ascertain if the seroprevalence of HSV-1, HSV-2, or rubella was increased, or higher geometric mean titers (GMTs) were observed more frequently among the subsets of CF patients who reported an acute onset of illness, had a docmented fever, or who met criteria for CFS, FM, or a psychiatric diagnosis.

PATIENTS AND METHODS

Subjects and Setting

The patient group was comprised of 548 consecutive individuals seen in the Chronic Fatigue Clinic at the University of Washington for an initial evaluation from November, 1988 until November, 1992. Patients completed the standardized clinic evaluation consisting of a comprehensive review of the current and past medical history, a physical examination, routine laboratory studies (complete blood count, erythrocyte sedimentation rate, automated chemical analysis, antinuclear antibodies, liver and thyroid function tests), and a structured psychiatric interview. Other studies were obtained as clinically indicated.

Although patients were not required to meet the Centers for Disease Control and Prevention (CDC) criteria for CFS [Holmes et al., 1988; Schluederberg et al., 1992] in order to be seen, data were systematically collected on each item in the case definition. The original 1988 CDC definition [Holmes et al., 1988] and the 1992 modification that clarifies the application of the criteria to those with psychiatric disorders were used to identify patients with CFS. Thus, most patients with non-psychotic psychiatric disorders who otherwise met criteria were classified as having CFS [Schluederberg et al., 1992]. Patients not fully meeting the criteria who also did not have evidence of a medical condition which could explain their fatigue, or previously documented schizophrenia, bipolar depression, or a personality disorder, were categorized as having unexplained chronic fatigue (referred to as CF). The majority of these patients did not have an adequate number of symptoms/signs to meet the criteria for CFS. The 1994 CDC case definition [Fukuda et al., 1994], which has similar psychiatric and medical exclusionary criteria as the 1988 modified version, was not used because it had not been formulated at the time this study was conducted and because it requires the collection of some data (e.g., patient height) that were not routinely obtained by our group (or others) at the time. The American College of Rheumatology criteria were used to diagnose FM [Wolfe et al., 1990].

Psychiatric diagnoses were determined using the National Institutes of Mental Health Diagnostic Interview Schedule Version III-A [APA, 1987]. This instrument is a highly structured interview that assigns current and lifetime diagnoses based on DSM III-R criteria [Robins and Helzer, 1985]. A version with sections on major depression, dysthymia, alcohol abuse, agoraphobia and somatization, generalized anxiety, and panic disorders was administered by a research assistant trained in its use. Patients were considered to have current major depression if criteria were met during the prior month.

Healthy control subjects were participants in a community-based study on CFS who explicitly denied fatigue and were not seeking medical care. Based on an extensive medical and psychological evaluation, these individuals were found to be without evidence of CFS or any other exclusionary medical or psychiatric condition. Similar to patients, healthy controls completed a questionnaire on their medical history, including all items required to diagnose CFS, and underwent the identical standardized physical examination and battery of blood tests. This study was approved by our human subjects office and written informed consent was obtained from both patients and controls.

Virologic Studies

Virologic studies for all 13 viruses were not obtained on each patient. During the first 3 months of the clinic's operation (November, 1988 through January, 1989), a comprehensive serologic evaluation was performed. However, preliminary analyses indicated that assays for HSV-1, HSV-2, rubella, and adenovirus did not differ from results in normal populations and clinically were not helpful in the evaluation of our patients. Thereafter, only HHV-6, EBV, CMV, and Coxsackie B antibody titers were obtained. Sera were obtained for viral serology during all seasons. Individual viral serologies were performed as follows: 1) Antibodies to HSV-1 and HSV-2 were detected by Western blot using previously validated methods for determining the presence of antibodies to HSV-1 and/or HSV-2 at a single serum dilution of 1:40 [Ashley et al., 1988]. 2) Antibodies to rubella were detected at a single dilution using a commercial ELISA (Abbott Laboratories, Chicago, IL). Specimens were considered positive according to the manufacturer's insert by comparing the reactivity of each specimen to that of control specimens provided with the kit. 3) Antibodies to adenovirus were assayed using complement fixation with serial dilutions from 1:2 to 1:32 [Hierholzer, 1989]. 4) Antibodies to HHV-6 were assayed using an ELISA and serial two-fold serum dilutions from 1:100 to 1:51,200. Endpoint titer determinations were made after further serial dilutions of sera that were still positive at the 51,200 dilution [Cone et al., 1993]. 5) Titers of IgM and IgG antibodies to EBV viral capsid antigen (VCA) and antibodies to the EBV early antigens (EA) were determined by indirect immunofluorescence using serial two-fold dilutions from 1:10 to 1:1280 for VCA IgG and EA antibodies [Andiman, 1989]. Anticomplement immunofluorescence was used to test for antibodies to nuclear antigens (EBNA) at a single 1:2 dilution. Reactivity above background signal obtained with EBNA-negative cells was considered positive [Andiman, 1989]. 6) End point titers of antibodies to CMV were determined by latex agglutination (Whittaker MA Bioproducts, Walkersville, MD) endpoint dilution using serial two-fold dilu-

TABLE I. Seroprevalence and Geometric Mean Titers of Viral Antibodies in Chronic Fatigue Patients and Controls[a]

Serology Result	Patients, n (%)	GMT	Controls, n (%)	GMT
HSV-1 positive	47/83 (57)	NA	13/20 (65)	NA
HSV-2 positive	14/83 (17)	NA	4/20 (20)	NA
Rubella positive	39/43 (91)	NA	19/20 (95)	NA
Adenovirus ≥64*	0/42 (0)	1.1	1/20 (5)	1.4**
HHV-6 ≥12,800*	39/295 (13)	3.3	2/30 (7)	3.1
EBV-VCA-IgG ≥640*	24/308 (8)	2.1	1/30 (3)	1.9
EBV-EA ≥80*	56/306 (18)	1.5	7/30 (23)	1.4
EBV-IgM ≥10*	2/310 (.6)	1.3	1/30 (3)	1.3
EBNA positive	293/308 (95)	NA	28/30 (93)	NA
CMV ≥2048*	9/285 (3)	2.2	1/30 (3)	1.8
Coxsackie B1 ≥256*	18/509 (3)	1.6	1/30 (3)	1.6
Coxsackie B2 ≥256*	42/509 (8)	1.8	4/30 (13)	1.7
Coxsackie B3 ≥256*	21/509 (4)	1.5	1/30 (3)	1.5
Coxsackie B4 ≥256*	31/509 (6)	1.6	3/30 (10)	1.6
Coxsackie B5 ≥256*	12/508 (2)	1.4	0/30 (0)	1.2
Coxsackie B6 ≥256*	1/509 (.2)	1.3	0/30 (0)	1.0
Any Coxsackie B1-6 >256	103/508 (20)	NA	8/30 (27)	NA

[a]NA, Not applicable; GMT, mean values after log transformation (base 10).
*Threshold values for normal results reported in control populations (see Methods section).
**$P < .0001$ patients vs. controls.

tions from 1:20 to 1:1024. 7) Antibody titers to Coxsackie B types 1 through 6 were ascertained by microneutralization of infectivity for buffalo green monkey cells and serial two-fold dilutions of sera from 1:8 to 1:512. Endpoint dilutions were determined by repeat testing of sera still positive at 1:512 [Grandien et al., 1989].

Statistical Analyses

All patients with CF were divided into subgroups based on meeting criteria for CFS, FM, or a current or lifetime psychiatric diagnosis, as well as the presence or absence of a self-reported onset of illness with an acute viral syndrome, or a temperature of >37.5°C on the intake physical examination. Patients not meeting criteria for CFS are referred to as the CF group.

Antibody titers were transformed using $\log_{10}$. Following confirmation of a normal distribution, GMTs were calculated using the $\log_{10}$ transformed data. Differences in normally-distributed continuous variables were examined using two-tailed tests and chi square analyses were used to compare proportions. Threshold levels for serology were chosen based on titers reported in control populations previously tested by the University of Washington Virology Laboratory and preliminary examination of the results of our control subjects using an upper limit of two standard deviations above the norm (upper 2.5%). These threshold values are shown in the left hand column of Table I. To adjust for differences in age and race between patients and controls, logistic regression was used for dichotomous variables (HSV-1, HSV-1, rubella, EBV EBNA) and multiple regression for continuous variables using GMTs as the dependent variable. Due to the large number of comparisons, only P values of <.01 were considered significant.

RESULTS

The 548 patients did not differ significantly from the 30 control subjects in the proportion of women (76% vs. 70%) or Caucasians (94% vs. 83%), but were significantly younger (mean age, 39.2 vs. 50.0 years, $P < .0001$). There were no consistent differences by age in the results of any viral titers. However, the absolute value of antibodies to EA ($P < .01$) and Coxsackie B1 GMTs ($P < .01$) varied in a non-linear fashion across decades.

Among all CF patients, the average duration of fatigue was 5.2 years. The illness commenced with a self-reported acute viral illness in 64%. Fifty-eight percent of the patients met the modified CDC criteria for CFS [Holmes et al., 1988; Schluederberg et al., 1992]; the remaining 42% reported chronic fatigue but did not meet the full CDC criteria. Not all of the 548 patients were tested for antibodies to each of the 13 viruses. However, there was no evidence of a selection bias in the patients tested: for each of the 13 viruses, there were no significant differences in age, gender, race, education, or duration of fatigue found in the subgroups of patients in whom serologies were and were not performed.

In general, the prevalence and GMTs of viral serologies did not differ between CF patients and control subjects for the 13 viruses tested (Table I). The GMT for adenovirus was significantly greater in control subjects than in the entire group of CF patients (1.4 vs. 1.1, $P < .0001$). With the exception of more positive results among women for HSV-1 ($P < .01$), there were no differences in antibodies between CF patients and control subjects for HSV-2, rubella, adenovirus, HHV-6, EBV IgG and EA, and Coxsackie B viruses 1 to 6 after adjusting for age, gender, and race using multiple regression techniques.

Our subsequent examination of subgroups found that viral serologies did not identify any special patient populations. In this regard, there were no differences observed between patients who met the case definitions for CFS or FM and those without these conditions (Table II). In addition, compared to the patient group that was

TABLE II. Viral Serology Results in Patients by Presence of Chronic Fatigue Syndrome and Fibromyalgia*

Serology	CFS Present		FM Present	
	Yes	No	Yes	No
HSV-1, n (%)	30/50 (60)	17/33 (52)	8/16 (50)	39/67 (58)
HSV-2, n (%)	11/50 (22)	3/33 (9)	4/16 (25)	10/67 (15)
Rubella, n (%)	24/26 (92)	15/17 (88)	6/6 (100)	33/37 (89)
Adenovirus (GMT)	1.1	1.1	1.1	1.1
HHV-6 (GMT)	3.3	3.4	3.4	3.3
EBV IgG (GMT)	2.1	2.1	2.1	2.1
EBV EA (GMT)	1.5	1.5	1.5	1.5
CMV (GMT)	2.2	2.2	2.1	2.2
Coxsackie B1 (GMT)	1.6	1.6	1.6	1.6
Coxsackie B2 (GMT)	1.8	1.8	1.7	1.8
Coxsackie B3 (GMT)	1.5	1.5	1.5	1.5
Coxsackie B4 (GMT)	1.6	1.6	1.6	1.6
Coxsackie B5 (GMT)	1.4	1.4	1.5	1.4
Coxsackie B6 (GMT)	1.3	1.2	1.4	1.2

*GMT, mean values after log transformation (base 10).

free of psychiatric illness, neither the presence of a current nor a lifetime disorder was associated with a higher GMT or frequency of positivity for the 13 viruses tested. Finally, results of viral serology did not differ among patients who reported that their illness commenced with an acute viral syndrome or in those with a documented fever compared to the groups without these characteristics.

DISCUSSION

Chronically fatiguing illnesses have been attributed to a variety of infectious agents. The viruses which have been the object of the most intensive scientific inquiry are the herpes viruses, including EBV [Straus et al., 1985; Holmes et al., 1987; Jones et al., 1985] and HHV-6 [Josephs et al., 1991; Buchwald et al., 1992], the enteroviruses [Bell et al., 1988; Calder et al., 1987; Halpin and Wessely, 1989; Yousef et al., 1988; Archard et al., 1988; Cunningham et al., 1990], particularly Coxsackie B, and a putative novel retrovirus [Khan et al., 1993]. However, since the demonstration of elevated antibody titers and other evidence of viral infection has been inconsistent, the abnormalities observed typically modest, and results not stratified by patient subgroups most likely to have viral involvement, the usefulness of viral serologies in CFS and CF has yet to be fully resolved. Early studies in the United States found increased levels of antibodies to EBV VCA and EA in patients with a CFS-like illness [Straus et al., 1985; Holmes et al., 1987; Jones et al., 1985]. Subsequent investigations of EBV and other herpes viruses, however, noted a large overlap in antibody titers between fatigued and healthy individuals, thus making EBV an unlikely etiologic agent in CFS or CF [Holmes et al., 1987; Horwitz et al., 1985; Lamy et al., 1982]. In an outbreak of a chronically fatiguing illness, we observed significantly higher GMTs of EBV VCA-IgG and a subset of antibodies to EA (EA-R) in cases compared to control subjects. Although antibody titers to HHV-6 were not significantly increased among patients, evidence of active replication of HHV-6 in primary lymphocyte cell cultures was demonstrated in 70% of these patients compared to 20% of the controls [Buchwald et al., 1992]. This observation underscores the fact that, at least for HHV-6 which produces a lytic infection of lymphocytes, antibody titers may not accurately reflect active viral replication [Buchwald et al., 1992; Ashley et al., 1988].

In the United Kingdom, several serologic surveys have found abnormal Coxsackie B serologies among individuals with "myalgic encephalomyelitis," an illness seemingly identical to CFS [Bell et al., 1988, Calder et al., 1987]. Two studies reported circulating complexes of Coxsackie viral antigen and IgM were present in most patients accompanied by isolation of enterovirus from stool in 22% [Halpin and Wessely, 1989; Yousef et al., 1988], results not replicated by others [Lynch and Seth, 1989]. Related findings reported by some [Archard et al., 1988; Cunningham et al., 1990] but not all [Gow et al., 1993] investigators have included the demonstration of enteroviral nucleic acid in muscle cells of chronically fatigued patients more often than in specimens obtained from control subjects.

In general, the studies cited above have examined only single viruses and did not examine subgroups of CFS and CF patients which might be more likely to have increased viral titers. To our knowledge, only two previous studies have sought serologic evidence for multiple viruses in these conditions. Besides our own study of herpes viruses cited above, Landay and colleagues [1991] examined the seroprevalence and GMTs of 10 viruses, including six reported in this study (EBV, CMV, HHV-6, Coxsackie B, adenovirus, and rubella), in several groups of fatigued patients. Findings were compared to healthy subjects recruited from outpatient clinics and laboratory personnel. Higher GMTs for antibodies to EBV EA, HHV-6, and Coxsackie B4, and lower GMTs for EBV EBNA were found among individuals meeting criteria for CFS. However, as in other studies, results were not stratified by potentially important patient characteristics such as a documented fever, an acute onset of illness

following a viral syndrome, or the presence of major depression. The study's findings were interpreted by the authors to reflect an underlying defect in T cell function rather than primary viral infection or clinically significant reactivation.

This interpretation is intriguing given the high prevalence of psychiatric disorders, particularly major depression in CFS and CF [Katon et al., 1991; Manu et al., 1991; Manu et al., 1988; Kruesi et al., 1989], and the demonstration that stressful life events, psychological distress, and affective illness are associated with immune dysfunction and elevations in antibody levels to viral agents [Glaser et al., 1985; Kiecolt-Glaser et al., 1987; Schleifer et al., 1989; Irwin et al., 1990]. Based on these observations, we postulated that patients with concurrent psychiatric diagnoses, particularly major depression, would be more likely to have elevated viral serologies than those without such disorders. Our findings, however, do not provide evidence for abnormalities in viral serologies among individuals with psychiatric illnesses, including those with depression. We also examined other subgroups in which evidence of increased viral activity might be likely such patients with subjective (self-reported acute onset with a viral syndrome) or objective (documented fever) evidence of infection, or those meeting strict criteria for CFS. Again, our results indicate that the virologic tests we studied are not useful in the indentification of any of these patient subgroups.

In conclusion, the current report extends observations that we and others have made regarding the limited usefulness of certain viral serologies in the evaluation of patients with CFS and CF. Our findings argue against the presence of a chronic infection but do not conclusively rule out a role for any of the viruses studied as etiologic agents or triggering events. Our data also do not support the hypothesis that CF with comorbid major depression is associated with serologic profiles consistent with viral reactivation suggestive of immune dysregulation. Although potentially helpful in evaluating individuals with signs, symptoms, or histories strongly suggestive of a specific virus, based on our findings, we do not recommend routinely obtaining the viral serologic tests evaluated in this investigation in chronically fatigued patients, including those who meet criteria for CFS, are febrile, or report an acute onset of illness.

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